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MOTILIN SYNTHETIC ANALOGUES AND MOTILIN RECEPTOR ANTAGONISTS

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While studying the structure-activity characteristics of motilin with motilin synthetic analogues, two compounds, motilin 1-12 $[CH_2\ NH]_{3-4}$ and motilin 1-12 $[CH_2\ NH]_{10-11}$, showed high affinity for the motilin receptor combined to a weak contractile activity. The following data suggest that motilin 1-12 $[CH_2\ NH]_{10-11}$ is a potent motilin receptor antagonist. It showed a high affinity for the motilin receptor present on membranes of rabbit antrum (pIC_{50}: 8.24 $\pm$ 0.08 for the analogue vs 8.96 $\pm$ 0.02 for the native peptide). When tested *in vitro* on strips of rabbit duodenum, the dose-response curve to motilin 1-22 was displaced to the right with motilin 1-12 $[CH_2\ NH]_{10-11}$ (pIC_{50}: 8.91 $\pm$ 0.06 in presence of saline versus 7.19 $\pm$ 0.40 with the analogue). However, when injected i.v. in dogs, motilin 1-12 $[CH_2\ NH]_{10-11}$ was undetectable in the peripheral blood, suggesting enzymatic degradation precluding its use *in vivo*.

Motilin is a 22 amino-acids polypeptide synthesized in endocrine cells of the duodeno-jejunal mucosa and present in plasma circulation of various mammals including dog and man. Motilin has been so named because of its biological potency to stimulate the motility of the gut (1). It is presumed that plasma motilin is an endocrine regulator of the interdigestive motility of the upper gut capable of inducing the phase III contraction of the migrating motor complex (MMC). Accumulated data strongly support this hypothesis in the dog (2, 3). In man however conclusive evidence for a physiological regulation by motilin of the interdigestive motility profile has still to be obtained (4, 5); some data also support the possibility that motilin could be active during the postprandial period in human (5-8).

Further progress in our understanding of canine or human motilin physiology will be achieved y blocking the biological action of the endogenous peptide with a specific antagonist. In order to develop specific antagonists to motilin, we undertook a detailed evaluation of the

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structure-activity characteristics of motilin (9-11). During this research program, where more than 120 analogues of motilin were synthesized, two of them presented a high affinity for the motilin receptor but a low contractile activity (11). These two products were then analyzed for their capacity to behave as motilin antagonists.

METHODOLOGY

Peptides: The analogues of motilin were synthesized by the solid phase procedure as previously described (9, 11). Two analogues of motilin, motilin 1-12 $[CH_2\ NH]_{3-4}$ and motilin 1-12 $[CH_2\ NH]_{10-11}$, showing high affinity for the motilin receptor combined to a weak contractile potency, appeared as good candidates for the current study (11).

Radioreceptor assay: The affinity of the two analogues for the motilin receptor was established in a radioreceptor assay evaluating the displacement of ^{125}I motilin 1-22 bound to membranes of rabbit antrum. (n = 4) This assay has been described earlier (11).

Bio-assay of motilin: The contractile activity of the two motilin analogues was tested *in vitro* (n = 4) by measuring the contraction of longitudinal strips of rabbit duodenum immersed in an organ bath (9). The contractile activity of porcine motilin or of motilin analogues was determined from doses ranging from 10^{-11} to 10^{-6} M and was compared to a maximum response to acetylcholine 10^{-5} M. For determination of the antagonistic capacity, the candidate analogue was added in the organ bath immediately before the dose-response curve to motilin 1-22 was established.

Bioavailability *in vivo*: This was realized in conscious dogs (n = 2) perfused with 75 pmol of motilin 1-22 or of motilin analogue $[CH_2NH]_{10-11}$. The 10 min infusion was given in a front limb vein and blood was obtained each 5 min during 30 min from a catheter installed in a rear limb vein. Plasma was frozen until assay by 7922 antiserum able to recognize both native motilin and motilin N-terminal fragments (10, 12).

Bioactivity *in vivo*: The contraction of the duodenum in response to a close intra-arterial injection of the motilin peptides was recorded in anaesthetized dogs (n = 2) as described before (13). Briefly, under anaesthesia maintained by iv pentobarbital, a laparotomy was performed to canulate the caudal pancreatic artery with a silastic tube continuously perfused with NaCl 0.9%. Strain gages (RB Products, Wisconsin) were sutured on the duodenum: one on the blanching area corresponding to the irrigation territory of the arterial canula, and two others were positioned respectively 10 cm orally and aborally to this zone. Strain gages were connected to a Beckman R611 recorder. Motilin 1-22 or/and motilin fragment $[CH_2NH]_{10-11}$ were injected intra-arterially (1 ml/30 s).

Data are presented as mean ±SEM. The amount of peptide required to obtain a contraction equivalent to 50% of the maximal response to acetylcholine (EC_{50}) or to displace 50% of the labelled motilin (IC_{50}) are referred in the text as the pEC_{50} and the pIC_{50} indicating the nagative logarithm of the original values.

RESULTS

Affinity to motilin receptor: When tested on membranes from rabbit antrum, $[CH_2NH]_{3-4}$ and $[CH_2NH]_{10-11}$ analogues were both able to displace ^{125}I motilin in a dose-response fashion. The pIC_{50} values were respectively of 6.80 ± 0.05 and 8.24 ± 0.08 for $[CH_2NH]_{3-4}$ and $[CH_2NH]_{10-11}$, as compared to a value of 8.96 ± 0.02 for the native peptide 1-22. (Fig. 1)

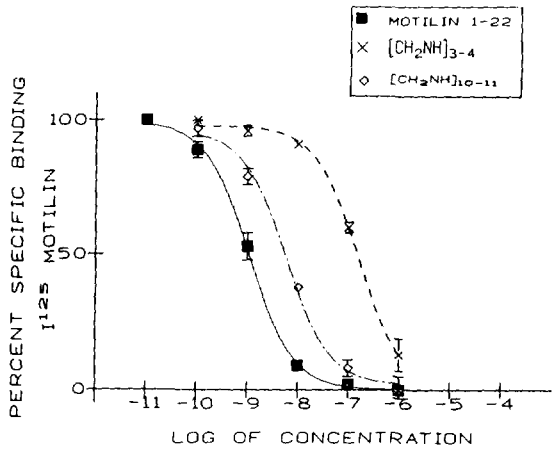

Figure 1. Displacement of ^{125}I motilin from rabbit antral membranes by the native peptide 1-22 and by motilin fragments 1-12 $[CH_2NH]_{3-4}$ and $[CH_2NH]_{10-11}$.

Bioactivity of analogues *in vitro*: $[CH_2NH]_{3-4}$ and $[CH_2NH]_{10-11}$ analogues were weak motilin agonists; both were able to elicit contraction of the duodenal strips when administered in very high concentrations (23% of maximal response at 10^{-6} M and 20% at 10^{-7} M respectively; data not shown). When they were tested against a background stimulation by motilin 1-22 (10^{-10} to 10^{-5} M), $[CH_2NH]_{3-4}$ (10^{-6} M) and $[CH_2NH]_{10-11}$ (10^{-7} M) displaced the dose-response curve to the right by approximatively 1 and 2 orders of magnitude respectively. The pEC_{50} value for motilin 1-22 administered with saline was 8.91 ± 0.06 and increased to 7.94 ± 0.07 and 7.19 ± 0.40 in the presence of $[CH_2NH]_{3-4}$ (10^{-6} M) or $[CH_2NH]_{10-11}$ (10^{-7} M). The response to

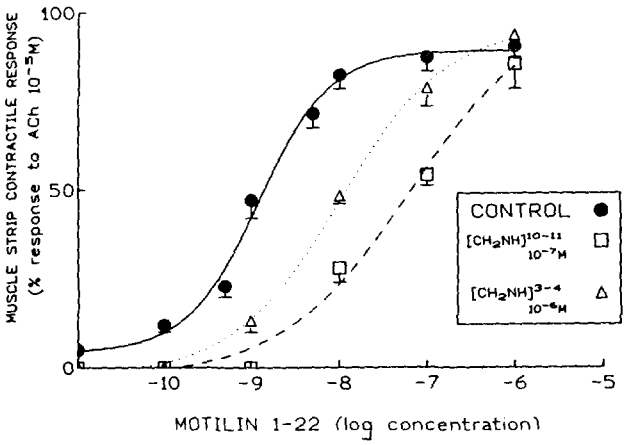

Figure 2. Contraction of rabbit duodenal strips in response to increasing doses of motilin 1-22 administered with saline (control) or with motilin fragments 1-12 $[CH_2NH]_{3-4}$ 10^{-6} M or $[CH_2NH]_{10-11}$ 10^{-7} M.

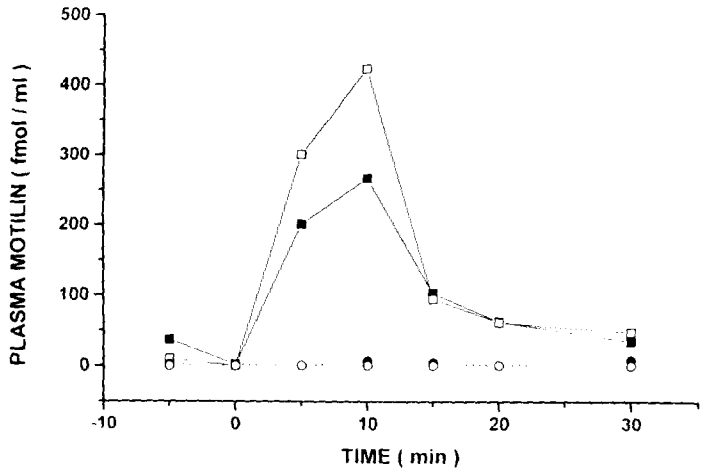

Figure 3. Increases in plasma motilin in 2 dogs (one in dark dots, and one in clear dots) following the iv administration of 75 pmol of motilin 1-22 (□——■) or of motilin 1-12 $[CH_2NH]_{10\text{-}11}$ (○- - - -●).

acetylcholine 10^{-5} M was not modified in the presence of motilin antagonists. These results are shown on fig. 2.

Bioavailability *in vitro*: Motilin 1-22 and motilin analogue $[CH_2NH]_{10\text{-}11}$ were both measurable by the N-terminus specific 7922 antiserum (ID 50 = 31.6 and 5.5 fmol/ml respectively). The motilin fragment 1-12 $[CH_2NH]_{3\text{-}4}$ was not detectable by the antiserum and could not therefore be tested *in vivo*. Plasma motilin raised by 331 ± 11 fmol/ml with the administration of motilin 1-22 and by only 2 ± 1 fmol/ml during infusion of $[CH_2NH]_{10\text{-}11}$ ($p < 0.001$). (Fig. 3)

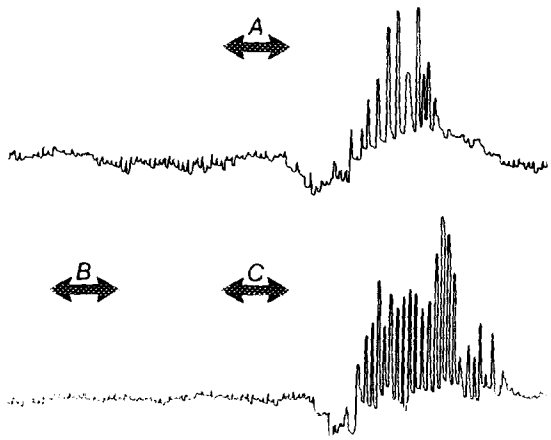

Figure 4. Contractile response of the canine duodenum in response to intra-arterial infusions of peptides given in 1 min indicated by the arrows. Motilin 1-12 $[CH_2NH]_{10\text{-}11}$ 0.5 10^{-5} M elicitated a small contraction (A). 10^{-6} M of the analogue was inactive (B) but it failed to inhibit the response to motilin 1-22 10^{-7} M (C).

Bioactivity *in vivo*: As previously described (13), motilin 1-22 elicitated a contractile response when injected in concentration of 10^{-7} M and above. The analogue $[CH_2NH]_{10-11}$ was without effect up to 10^{-6} M, but doses of 0.5 10^{-5} M and 10^{-5} M induced a contractile response of the duodenum. $[CH_2NH]_{10}$ (10^{-6} M) was then tested as an antagonist but it failed to modify the contraction elicitated by motilin 1-22 10^{-7} M. (Fig.4)

DISCUSSION

We have synthesized two motilin analogues that have a high affinity for the motilin receptor and that can block the contractile effect of motilin 1-22 in the rabbit duodenum *in vitro*. When tested *in vivo*, the $[CH_2NH]_{10-11}$ compound was unstable and undetectable in the peripheral circulation when injected intravenously.

The importance of developing a motilin antagonist for further studies on the regulation of gastrointestinal motility is obvious, but, up to now, such a product has not been available. Erythromycin derivatives, also designated as macrolides, have been developed as potent motilin receptor agonists (14, 15) but, to our knowledge, this approach has failed to provide any specific antagonist. T.L. Peeters in Belgium has explored, as we did, the possibility of developing a motilin receptor antagonist from motilin synthetic analogues. They have recently published their data concerning one compound (ANQ-111) that could behave like a motilin agonist at high concentration (10^{-5} M) and that could decrease the dose-response curve of motilin 1-22 by about one order of magnitude when tested at 10^{-6} M on strips of rabbit duodenum *in vitro* (16). The structure of this compound has not been disclosed, and its bioavailability or its efficiency *in vivo* have not been revealed. The antagonist that we report here seems slightly more potent than the one published by the Belgium group.

The lack of bioavailability of motilin 1-12 $[CH_2NH]_{10-11}$, when administered in the peripheral circulation, was not unexpected considering our previous results obtained with N-terminal motilin fragments *in vivo* and suggesting that the C-terminal portion of the molecule was probably essential to preserve the molecule against enzymatic degradation *in vivo* (10). In the experimental model with the close intra-arterial injection procedure, the motor response induced by motilin 1-12 $[CH_2NH]_{10-11}$ confirmed that the product was active on the canine motilin receptor, although it seemed to behave as a weak agonist. The contraction was obtained with doses 50-100 times greater than with the native molecule. However, the affinity of motilin 1-12 $[CH_2NH]_{10-11}$ for the canine motilin receptor *in vivo* remains undetermined since our experimental protocol did not allow to verify the residual bioavailability of the compound in this model.

Our previous studies on the structure-activity of motilin taught us that the biological activity of the molecule resides in the first 7 amino-acids (9, 11) and that this N-terminal portion

probably needs to stay in a non restrictional conformation (11). We also learned that the binding of the peptide to its receptor is probably facilitated by a C-terminal alpha helical structure initiated from the middle portion of the molecule (11), and that this middle portion of motilin is probably important to provide protection of the molecule against some enzymatic degradation that could occur when injected intravenously (10). Additional work is required to develop motilin receptor antagonists that could have a very high affinity for the receptor while being deprived of any biological stimulation, and that could be used *in vivo*. The data we presented here, and those recently published by Peeters' group (16), clearly suggest that such an antagonist could be available soon.

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